Current Biology, Vol. 15, 2021–2026, November 22, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.10.040

Fungal Adenylyl Cyclase Integrates CO_2 Sensing with cAMP Signaling and Virulence

Torsten Klengel,[1,6] Wei-Jun Liang,[1,6] James Chaloupka,[2,6] Claudia Ruoff,[1] Klaus Schröppel,[3,7] Julian R. Naglik,[4] Sabine E. Eckert,[1] Estelle Gewiss Mogensen,[1] Ken Haynes,[5] Mick F. Tuite,[1] Lonny R. Levin,[2] Jochen Buck,[2,*] and Fritz A. Mühlschlegel[1,*]

[1] Department of Biosciences
University of Kent
Canterbury, Kent CT2 7NJ
United Kingdom

[2] Department of Pharmacology
Joan and Sanford I. Weill Medical College of Cornell University
New York, New York 10021

[3] Institut für Klinische Mikrobiologie, Immunologie, und Hygiene
Universität Erlangen
91054 Erlangen
Germany

[4] Department of Oral Medicine, Pathology, and Immunology
GKT Dental Institute
King's College London
London, SE1 9RT
United Kingdom

[5] Department of Infectious Diseases
Imperial College London
London, W12 ONN
United Kingdom

Summary

The ascomycete *Candida albicans* is the most common fungal pathogen in immunocompromised patients [1]. Its ability to change morphology, from yeast to filamentous forms, in response to host environmental cues is important for virulence [2–5]. Filamentation is mediated by second messengers such as cyclic adenosine 3′,5′-monophosphate (cAMP) synthesized by adenylyl cyclase [4]. The distantly related basidiomycete *Cryptococcus neoformans* is an encapsulated yeast that predominantly infects the central nervous system in immunocompromised patients [6–8]. Similar to the morphological change in *C. albicans*, capsule biosynthesis in *C. neoformans*, a major virulence attribute, is also dependent upon adenylyl cyclase activity [7]. Here we demonstrate that physiological concentrations of CO_2/HCO_3^- induce filamentation in *C. albicans* by direct stimulation of cyclase activity. Furthermore, we show that CO_2/HCO_3^- equilibration by carbonic anhydrase is essential for pathogenesis of *C. albicans* in niches where the available CO_2 is limited. We also demonstrate that adenylyl cyclase from *C. neoformans* is sensitive to physiological concentrations of CO_2/HCO_3^-. These data demonstrate that the link between cAMP signaling and CO_2/HCO_3^- sensing is conserved in fungi and reveal CO_2 sensing to be an important mediator of fungal pathogenesis. Novel therapeutic agents could target this pathway at several levels to control fungal infections.

*Correspondence: jobuck@med.cornell.edu (J.B.); f.a.muhlschlegel@kent.ac.uk (F.A.M.)

[6] These authors contributed equally to this work.

[7] Present address: Robert-Bosch-Hospital, 70376 Stuttgart, Germany.

Results and Discussion

CO_2 Is a Powerful Inducer of Filamentation that Requires Adenylyl Cyclase, Bypasses Ras, and Is Independent of *C. albicans* Aquaporin

Much of the success of *C. albicans* as a pathogen is attributed to its ability to adapt to the diverse microbial habitats in the mammalian host. Indeed, various mammalian environmental cues (i.e., pH of 7.4, an ambient temperature of 37°C, serum) induce a reversible transition from yeast-like to filamentous growth form that is critical for virulence [2–5]. Several regulators of this highly controlled developmental program have been identified. For example, filamentation in response to serum involves Ras activation of the adenylyl cyclase/cAMP-signaling pathway [4, 9, 10]. In fact, in *C. albicans,* adenylyl cyclase (AC) is essential for morphogenesis in response to all known physiological signals [4].

In contrast, involvement of a critical physiological signal, CO_2 concentration, has not been investigated in the pathogenesis of *C. albicans* [11]. In mammals, the CO_2 concentration is more than 150-fold higher (5%) than it is in atmospheric air (0.033%) [12, 13]. Consequently, fungal pathogens, including *C. albicans,* are exposed to dramatically different CO_2 concentrations during superficial infections compared with invasion of the blood. We found that 5% CO_2 induces filamentation, predominantly in the form of pseudohyphal development, or invasion of the underlying agar in 208 independent isolates of *C. albicans* (Figures 1A and 1B) but not other yeast species (51 *Candida dubliniensis*, 45 *Candida glabrata*, 22 *Candida parapsilosis*, 11 *Candida krusei*, and 4 *Saccharomyces cerevisiae*) [14]. *C. albicans* filamentation in response to CO_2 was observed on seven different media (YNB, YEPD, chocolate agar, Columbia blood agar, medium M199, and DMEM), including a matrix made up of distilled water and 2% agar, and it was independent of pH; *C. albicans* responded to 5% CO_2 at both pH 7 and pH 4 although filamentation rates were higher at pH 7 (80% at pH 7 versus 30% at pH 4). These observations are consistent with the findings of Mock et al. [11] who demonstrated that 25 mM bicarbonate induces filamentation in *C. albicans*.

To determine if CO_2-mediated agar invasion and filamentation were dependent on signaling proteins known to govern filamentation in response to other stimuli, i.e., Ras or AC, we exposed mutant strains of *C. albicans* that lacked either *RAS1* or the AC-encoding gene *CDC35* to 5% CO_2 [4, 9]. Exposure to 5% CO_2 induced the *ras1Δ/ras1Δ* strain to invade the underlying agar and form a filamentous colony fringe (Figures 1B and 1C). In contrast,

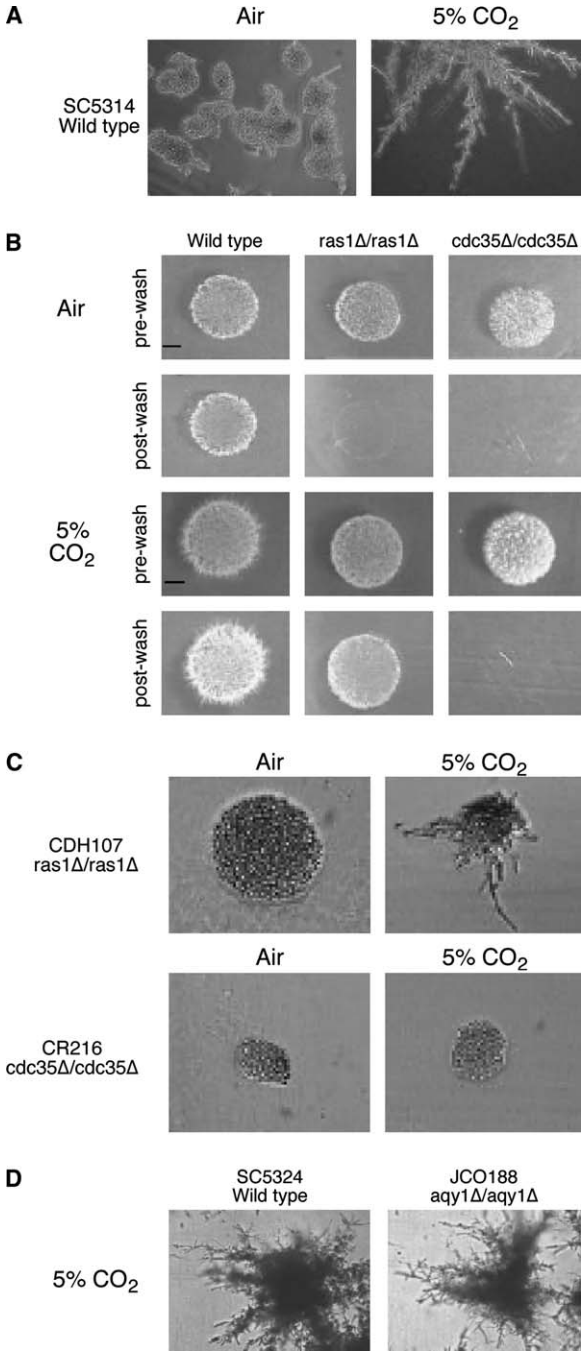

Figure 1. CO_2 Sensing Requires Adenylyl Cyclase, Bypasses Ras, and Is Independent of *C. albicans* Aquaporin

(A) Wild-type strain SC5314 grown on DMEM medium (pH 7) in air (left) or 5% CO_2 (right). Cells were incubated for 24 hr at 37°C and photographed at ×70 magnification. The pH of the medium was adjusted to pH 7.0 using 150 mM HEPES as previously described [25, 26].

(B) 1×10^5 *C. albicans* cells from strains SC5314, CDH107 [6] (*ras1Δ/ras1Δ*), and CR216 [4] (*cdc35Δ/cdc35Δ*) were spotted onto DMEM medium. Cells were incubated for 48 hr at 37°C in either air (top 2 rows) or 5% CO_2 (bottom 2 rows). Cells were photographed before and after washing. Scale bars equal 1 mm.

(C) *C. albicans* cells from strains CDH107 (*ras1Δ/ras1Δ*) (top) and CR216 (*cdc35Δ/cdc35Δ*) (bottom) grown on DMEM medium. Cells were incubated for 12 hr at 37°C in either air or 5% CO_2 and photographed at ×70 magnification.

(D) Wild-type strain SC5314 (left) and JCO188 (*aqy1Δ/aqy1Δ*, right) [20] were grown on DMEM medium in 5% CO_2. Cells were incubated for 24 hr at 37°C and photographed at ×70 magnification.

the *cdc35Δ/cdc35Δ* strain lacking AC was refractory to 5% CO_2 and failed to invade the agar or filament (Figures 1B and 1C). Thus, CO_2 signaling in *C. albicans* bypasses Ras but requires AC.

In mammalian cells, cAMP is synthesized by transmembrane adenylyl cyclases (tmACs) and soluble adenylyl cyclase (sAC) [15]. In contrast to tmAC, sAC is activated by physiological concentrations of bicarbonate [15, 16]. Bicarbonate-responsive sAC-like ACs are also found in bacteria [15], revealing the link between bicarbonate sensing and the second messenger cAMP to be among the most evolutionarily ancient signaling pathways. Bicarbonate stimulation of mammalian and bacterial sAC-like cyclases is direct [15] and works by inducing a conformational change, which facilitates catalysis but has little effect on affinity for substrate ATP [17].

Transport of CO_2 across biological membranes by aquaporin water channels has been reported for both plant and mammalian cells [18]. *C. albicans* contains a single aquaporin gene (*AQY1*) that encodes a functional water channel [19]. To determine if Aqy1 was required for CO_2-mediated polymorphism, we exposed the *C. albicans aqy1Δ/aqy1Δ* mutant to 5% CO_2. We found that this strain was not restricted in its ability to filament compared to wild-type (Figure 1D).

Nce103 Is a Carbonic Anhydrase that Is Essential for Pathological Growth in Niches where Sufficient CO_2 Is Not Supplied by the Host

Cellular effects of CO_2 can be mediated via its hydrated form, bicarbonate. Bicarbonate is spontaneously formed from CO_2 in solution, but this reaction is greatly accelerated by a ubiquitous family of carbonic anhydrases. We cloned the *C. albicans NCE103* gene showing similarities to *NCE103* from *S. cerevisiae* encoding a β-class carbonic anhydrase [20]. Stopped-flow experiments with purified *C. albicans* Nce103 demonstrated carbonic anhydrase activity of the protein and confirmed inhibition by the carbonic anhydrase inhibitor ethoxyzolamide (Figure 2A).

To investigate the role of Nce103 in growth and virulence, we deleted *NCE103* in two separate strain backgrounds (CAI4 and BWP17). Mutant strains in either background showed similar phenotypes. *C. albicans* TK1 and TK1a (*nce103Δ/nce103Δ* in a CAI4 or BWP17 background) grew at 5% CO_2 but failed to grow in air (Figure 2B and Supplemental Data available with this article online). Reintroduction of a single copy of *C. albicans NCE103*, yielding TK2 and TK2a (*nce103Δ/nce103Δ* + *NCE103*), restored the ability to grow and filament in air. We also found that *C. albicans* senses CO_2 over a range of concentrations and that 0.5% already partially complemented the growth defect of TK1 (Supplemental Data).

The inability of TK1 to grow in air may be due to limiting amounts of bicarbonate known to serve as a substrate for several cellular carboxylases important to metabolism. However, addition of various cellular metabolites and carbon sources, including citrate, succinate, oxalacetate, malate, α-ketoglutarate, arginine, adenine,

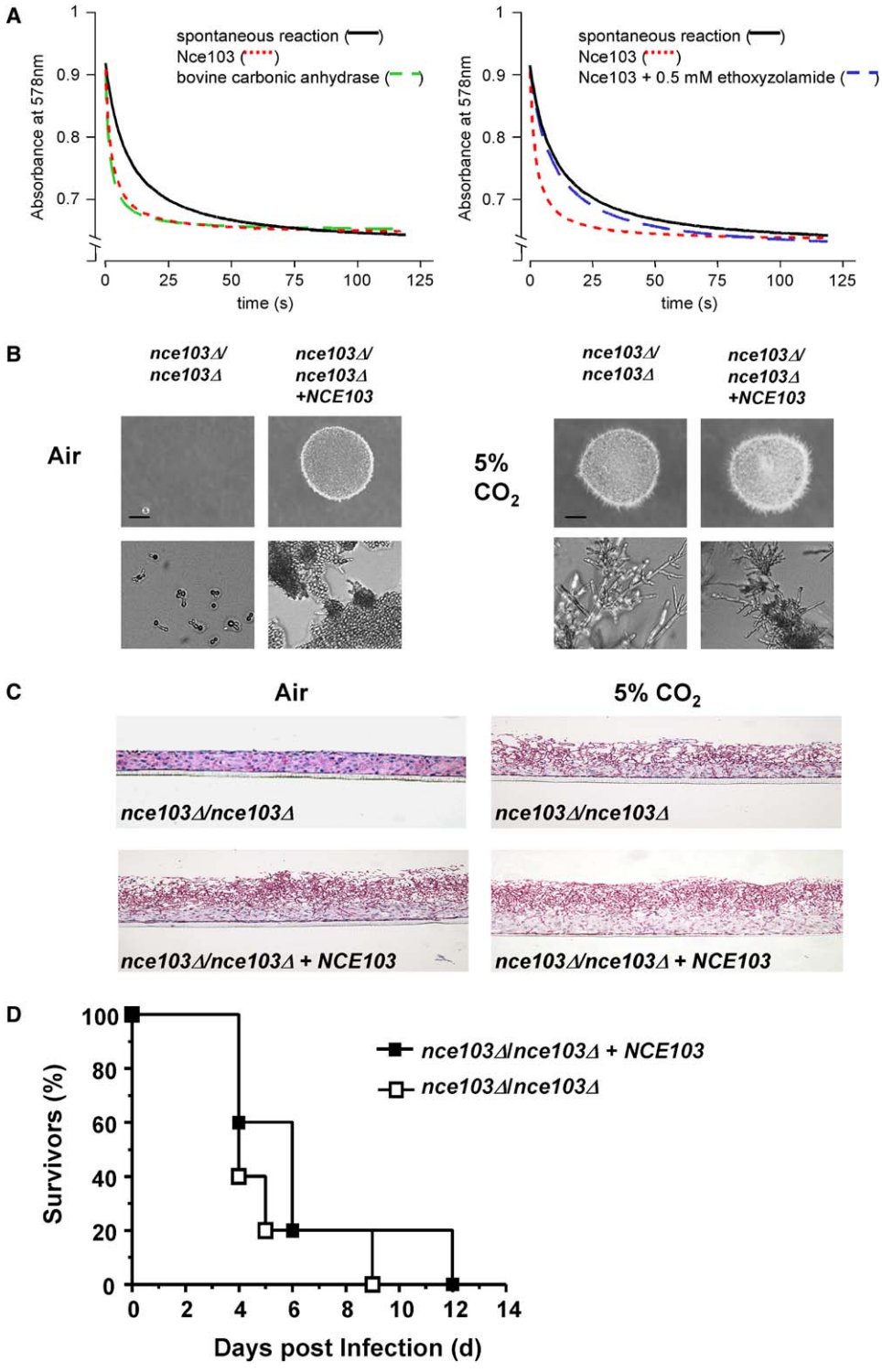

Figure 2. Nce103 Is a Carbonic Anhydrase that Is Required for Atmospheric Pathogenicity

(A) Biochemical analysis of Nce103 function by stopped flow. Left: spontaneous chemical hydration of CO_2 to bicarbonate (black line); reaction catalyzed by Nce103 (red line); reaction catalyzed by bovine carbonic anhydrase (green line). Right: spontaneous chemical hydration of CO_2 to bicarbonate (black line); reaction catalyzed by Nce103 (red line); inhibition of *C. albicans* Nce103 activity by 0.5 mM ethoxyzolamide (blue line). The reaction from CO_2 to HCO_3^- imposes a decrease of the pH. The pH-sensitive indicator m-cresol purple changes color from purple to yellow and therefore the absorption at 578 nm decreases. The absorbance in the catalyzed reaction decreases faster than in the noncatalyzed reaction.

(B) *C. albicans* TK1 (*nce103Δ/nce103Δ*) and reconstituted TK2 (*nce103Δ/nce103Δ + NCE103*) were either spotted (1×10^5 cells) (top) or streaked (bottom) onto DMEM medium and incubated in air (left four panels) or 5% CO_2 (right four panels) at 37°C for 48 hr. Streaked cells were photographed at ×70 magnification. Scale bars equal 1 mm.

and oleate, failed to complement the growth defect of TK1 (Supplemental Data and data not shown). Interestingly, in an accompanying paper in this issue of *Current Biology*, Bahn et al. succeeded in complementing the growth defect of a *C. neoformans* carbonic anhydrase mutant with the fatty acid palmitate [27].

CO_2 concentrations in skin will be lowered due to equilibration with the atmosphere, yet fungi including *Candida* must exploit these lower levels during epithelial infections. Consistently, elevating CO_2 concentrations at the skin surface of patients have been reported to aggravate *Candida* infections [21]. When tested in an experimental model of atmospheric pathogenicity using human oral epithelium, the *nce103Δ/nce103Δ* mutant failed to inflict damage to the epithelium, while the control strain, with a single copy of *C. albicans NCE103* (*nce103Δ/nce103Δ* + *NCE103*), invaded the epithelium (Figure 2C). When the experiment was repeated in an atmosphere containing 5% CO_2, both the *nce103Δ/nce103Δ* null mutant and the reconstituted strain exhibited a comparable degree of tissue damage (Figure 2C). We also tested the carbonic anhydrase mutants in an intravenous model of systemic candidiasis, in which *Candida* is exposed to the higher partial pressures of CO_2 present in blood. Injection of either control (TK2) or mutant (TK1) strain resulted in death of 50% of mice by day 4 and 100% by day 9 or day 12 postinfection (Figure 2D); thus, there was no discernible difference due to the absence of *NCE103*. Further comparison of TK1 and TK2 in two additional CO_2-rich host niches (a pulmonary model of invasive candidiasis in immunosuppressed mice and a vaginal model of candidiasis) did not reveal any virulence defects of the *nce103Δ/nce103Δ* mutant TK1 (Supplemental Data). Therefore, not only can CO_2 function as a differentiation factor for *C. albicans,* but its conversion to bicarbonate via carbonic anhydrase is essential for causing damage in niches where sufficient CO_2 is not supplied by the host.

The Catalytic Domain of *C. albicans* AC Is Sufficient to Induce CO_2-Mediated Filamentation

To explore whether the link between CO_2 and the AC might be direct, we expressed either the entire *CDC35* coding region or a fragment encoding amino acids 1166–1571, containing the presumptive catalytic domain, under the control of the *TEF2* promoter in the *cdc35Δ/cdc35Δ* strain CR276. Expression of either full-length or truncated Cdc35 restored CO_2-mediated filamentation in CR276 (Figure 3A). Thus, the Cdc35 catalytic domain is sufficient to support CO_2 sensing.

C. neoformans AC Restores the Ability to Sense CO_2 in the *C. albicans cdc35Δ/cdc35Δ* Mutant

A signal capable of inducing capsule biosynthesis in *C. neoformans* is elevated carbon dioxide (CO_2), but the link between this ubiquitous by-product of cellular metabolism and *C. neoformans* AC has remained unknown [8]. To determine whether CO_2 sensing is conserved in fungal pathogens, we expressed the catalytic

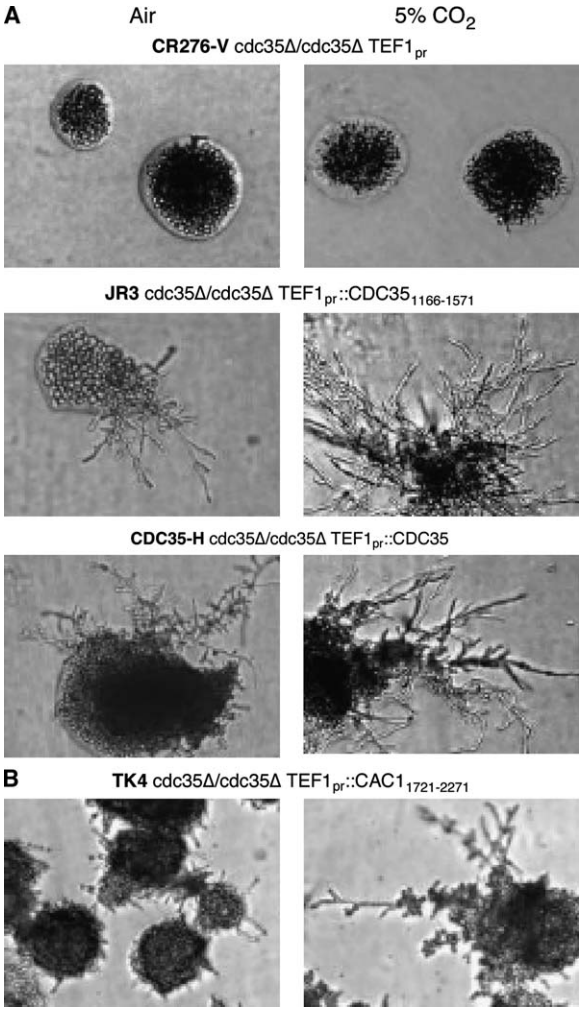

Figure 3. Truncated Adenylyl Cyclase Restores CO_2 Sensing

C. albicans CR276-V (*cdc35Δ/cdc35Δ* + *TEF2pr*); JR-3 (*cdc35Δ/cdc35Δ* + *TEF2pr::CDC351166-1571*) expressing truncated Cdc35; CDC35-H (*cdc35Δ/cdc35Δ* + *TEF2pr::CDC35*) expressing full-length Cdc35 (A), and TK4 (*cdc35Δ/cdc35Δ* + *TEF2pr::CAC11721-2271*) expressing truncated *C. neoformans* Cac1 (B) were grown on DMEM (bottom two images) medium in air (left column) or 5% CO_2 (right column). Cells were incubated for 24 hr at 37°C and photographed at ×70 magnification.

domain of the *C. neoformans* AC [7] in the *C. albicans cdc35Δ/cdc35Δ* strain. A *CAC1* fragment encoding amino acids 1721–2271 restored the ability of the *C. albicans cdc35Δ/cdc35Δ* strain to filament upon exposure to 5% CO_2 (Figure 3B). Thus, ACs from other, distantly related, fungi can restore the ability to sense CO_2, suggesting that the link between cAMP and CO_2 sensing may be a general feature of pathogenic fungi.

ACs from *C. albicans* and *C. neoformans* Are CO_2 Chemosensors

We previously demonstrated that mammalian and bacterial sAC-like cyclases are directly stimulated by

(C) Human reconstituted epithelium was infected with *C. albicans* TK1 (*nce103Δ/nce103Δ*) and reconstituted TK2 (*nce103Δ/nce103Δ* + *NCE103*) and incubated in air (left) or 5% CO_2 (right) at 37°C for 24 hr and photographed at ×100 magnification. *C. albicans* TK1 can invade the epithelium only in the presence of 5% CO_2, while TK2 causes tissue damage in air as well as 5% CO_2.
(D) Balb/c mice (n = 5) were intraveneously infected with 2×10^5 viable blastoconidia of TK1 (white squares) and TK2 (black squares).

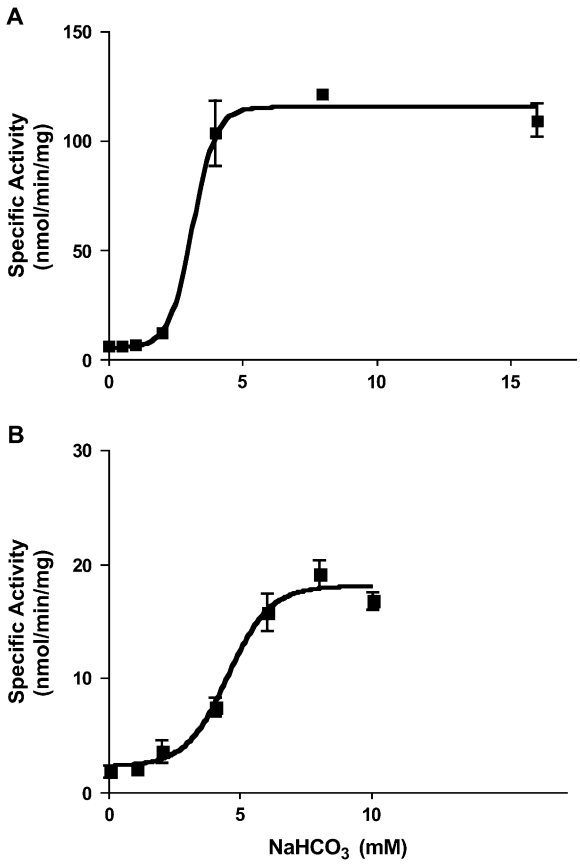

Figure 4. Bicarbonate Activates *C. albicans* and *C. neoformans* Adenylyl Cyclases

Purified *C. albicans* $Cdc35_{1166-1571}$ (A) and *C. neoformans* $Cac1_{1721-2271}$ (B) were assayed in the presence of the indicated concentrations of $NaHCO_3$ with 10 mM ATP and 40 mM $MgCl_2$. Data are expressed as picomoles of cAMP formed per minute per milligram of protein, and values are averages of triplicate determinations. Shown are representative experiments repeated at least three times. Error bars indicate standard error from the mean (SEM).

physiological levels of bicarbonate [15], but fungal ACs, including the *C. albicans* and *C. neoformans* cyclases, are only modestly related to sAC-like ACs [16]. To test whether fungal cyclases might also directly sense CO_2/HCO_3^-, we purified recombinant *C. albicans* Cdc35 and *C. neoformans* Cac1 proteins. Purified *C. albicans* cyclase was stimulated more than 20-fold with a median effective concentration (EC_{50}) of 3.5 ± 0.3 mM bicarbonate (Figure 4A), and *C. neoformans* AC was stimulated nearly 2-fold with an EC_{50} of 4.1 ± 0.1 mM bicarbonate (Figure 4B). These dose-response relationships reveal why 5% CO_2, which corresponds to ~25 mM bicarbonate (at pH 7.4) and would maximally stimulate both fungal ACs, was sufficient to induce the filamentous transition in *C. albicans*. In contrast, in skin where diffusion into the atmosphere results in significantly lower endogenous CO_2 concentrations, the *C. albicans* cyclase would require carbonic anhydrase activity to achieve sufficiently high bicarbonate concentrations to support its activity. When measuring intracellular cAMP changes in the presence and absence of CO_2 with a number of different protocols, we found that the resting intracellular cAMP levels were extremely low, and the CO_2-induced changes we observed were too small to be statistically significant (data not shown).

Bicarbonate regulation of cAMP synthesis provides a mechanism for the CO_2-dependent filamentation in *C. albicans* and CO_2-dependent capsule biosynthesis in *C. neoformans*. Morphogenesis in the fungal pathogen *Coccidioides immitis* is positively regulated by physiological CO_2 concentrations [22], suggesting that this fungal pathogen may also be dependent upon a bicarbonate-regulated AC for virulence. Conservation among distantly related fungi, along with the presence of bicarbonate-sensitive ACs in animals and prokaryotes [15, 16, 23], establish CO_2/HCO_3^- chemosensing via the second messenger cAMP as one of the most ancient and widely conserved signaling pathways in biology. Furthermore, our finding that a carbonic anhydrase is essential for growth and virulence in specific niches where the CO_2 concentration is limiting, in conjunction with reports that assign a role in virulence to bacterial carbonic anhydrase (*Salmonella enteritidis*) [24], identifies the CO_2-chemosensing pathway as a prime target for the development of new antimicrobial agents.

Supplemental Data

Supplemental Data include two figures and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/15/22/2021/DC1/.

Acknowledgements

We thank Jo Roobol and Alexander J. Woodman for their help throughout this study. We would like to thank P. Agre, E. Leberer, M. Whiteway, G.R. Fink, and W.A. Fonzi for strains and Mike Geeves and David Pearson for suggestions and help with stopped-flow experiments. The Wellcome Trust (to F.A.M.), BBSRC (to F.A.M.), EU (to F.A.M.), NIH (to J.B. and L.R.L.), Ellison Medical Foundation (to J.B.), and Hirschl/Weill-Caulier Foundation (to L.R.L.) provided financial support. T.K. was supported by a predoctoral fellowship from the Studienstiftung des Deutschen Volkes. The authors have no conflicting financial interests. We would like to thank the three anonymous reviewers for their constructive comments on this work.

Received: August 11, 2005
Revised: September 26, 2005
Accepted: October 12, 2005
Published: November 21, 2005

References

1. Fridkin, S., and Jarvis, W.R. (1996). Epidemiology of nosocomial fungal infections. Clin. Microbiol. Rev. *9*, 499–511.
2. Brown, A.J., and Gow, N.A. (1999). Regulatory networks controlling *Candida albicans* morphogenesis. Trends Microbiol. *7*, 333–338.
3. Lo, H.J., Kohler, J.R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., and Fink, G.R. (1997). Nonfilamentous *C. albicans* mutants are avirulent. Cell *90*, 939–949.
4. Rocha, C.R., Schroppel, K., Harcus, D., Marcil, A., Dignard, D., Taylor, B.N., Thomas, D.T., Whiteway, M., and Leberer, E. (2001). Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus *Candida albicans*. Mol. Biol. Cell *12*, 3631–3643.
5. De Bernardis, F., Muhlschlegel, F.A., Cassone, A., and Fonzi, W.A. (1998). The pH of the host niche controls gene expression in and virulence of *Candida albicans*. Infect. Immun. *66*, 3317–3325.
6. Janbon, G. (2004). *Cryptococcus neoformans* capsule biosynthesis and regulation. FEMS. Yeast Res. *4*, 765–771.
7. Alspaugh, J.A., Pukkila-Worley, R., Harashima, T., Cavallo, L.M., Funnell, D., Cox, G.M., Perfect, J.R., Kronstad, J.W., and

Heitman, J. (2002). Adenylyl cyclase functions downstream of the G alpha protein and controls mating and virulence of *Cryptococcus neoformans*. Eukaryot. Cell *1*, 75–84.

8. Granger, D.L., Perfect, J.R., and Durack, D.T. (1985). Virulence of *Cryptococcus neoformans*. Regulation of capsule synthesis by carbon dioxide. J. Clin. Invest. *76*, 508–516.
9. Leberer, E., Harcus, D., Dignard, D., Johnson, L., Ushinsky, S., Thomas, D.Y., and Schroppel, K. (2001). Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus *Candida albicans*. Mol. Microbiol. *42*, 673–687.
10. Feng, Q., Summers, E., Guo, B., and Fink, G.R. (1999). Ras signalling is required for serum-induced hyphal differentiation in *Candida albicans*. J. Bacteriol. *181*, 6339–6346.
11. Mock, R.C., Pollack, J.H., and Hashimoto, T. (1990). Carbon dioxide induces endotrophic germ tube formation in *Candida albicans*. Can. J. Microbiol. *36*, 249–253.
12. Guyton, A.C., and Hall, J.E. (2000). Textbook of Medical Physiology (Philadelphia, PA: W.B. Saunders).
13. Monnin, E., Indermuhle, A., Dallenbach, A., Fluckiger, J., Stauffer, B., Stocker, T.F., Raynaud, D., and Barnola, J.M. (2001). Atmospheric CO_2 concentrations over the last glacial termination. Science *291*, 112–114.
14. Sheth, C., Johnson, E.J., Baker, M.E., Haynes, K., and Mühlschlegel, F.A. (2005). Phenotypic identification of *Candida albicans* by growth on chocolate agar. Med. Mycol., in press.
15. Chen, Y., Cann, M.J., Litvin, T.N., Lourgenko, V., Sinclair, M.L., Levin, L.R., and Buck, J. (2000). Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science *289*, 625–628.
16. Buck, J., Sinclair, M.L., Schapal, L., Cann, M.J., and Levin, L.R. (1999). Cytosolic adenylyl cyclase defines a unique signalling molecule in mammals. Proc. Natl. Acad. Sci. USA *96*, 79–84.
17. Steegborn, C., Litvin, T.N., Levin, L.R., Buck, J., and Wu, H. (2005). Bicarbonate activation of adenylyl cyclase via promotion of catalytic active site closure and metal recruitment. Nat. Struct. Mol. Biol. *12*, 32–37.
18. Uehlein, N., Lovisolo, C., Siefritz, F., and Kaldenhoff, R. (2003). The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature *425*, 734–737.
19. Carbrey, J.M., Cormack, B.P., and Agre, P. (2001). Aquaporin in *Candida*: characterization of a functional water channel protein. Yeast *18*, 1391–1396.
20. Amoroso, G., Morell-Avrahov, L., Muller, D., Klug, K., and Sultemeyer, D. (2005). The gene *NCE103* (*YNL036w*) from *Saccharomyces cerevisiae* encodes a functional carbonic anhydrase and its transcription is regulated by the concentration of inorganic carbon in the medium. Mol. Microbiol. *56*, 549–558.
21. Allen, A.M., and King, R.D. (1978). Occlusion, carbon dioxide, and fungal skin infections. Lancet *1*, 360–362.
22. Klotz, S.A., Drutz, D.J., Huppert, M., Sun, S.H., and DeMarsh, P.L. (1984). The critical role of CO_2 in the morphogenesis of *Coccidioides immitis* in cell-free subcutaneous chambers. J. Infect. Dis. *150*, 127–134.
23. Cann, M.J., Hammer, A., Zhou, J., and Kanacher, T. (2003). A defined subset of adenylyl cyclases is regulated by bicarbonate ion. J. Biol. Chem. *278*, 35033–35038.
24. Valdivia, R.H., and Falkow, S. (1997). Fluorescence-based isolation of bacterial genes expressed within host cells. Science *277*, 2007–2011.
25. Muhlschlegel, F.A., and Fonzi, W.A. (1997). *PHR2* of *Candida albicans* encodes a functional homolog of the pH-regulated gene *PHR1* with an inverted pattern of pH-dependent expression. Mol. Cell. Biol. *17*, 5960–5967.
26. El Barkani, A., Kurzai, O., Fonzi, W.A., Ramon, A., Porta, A., Frosch, M., and Muhlschlegel, F.A. (2000). Dominant active alleles of *RIM101* (*PRR2*) bypass the pH restriction on filamentation of *Candida albicans*. Mol. Cell. Biol. *20*, 4635–4647.
27. Bahn, Y.-S., Cox, G.M., Perfect, J.R., and Heitman, J. (2005). Carbonic anhydrase and CO_2 sensing during *Cryptococcus neoformans* growth, differentiation, and virulence. Curr. Biol. *15*, this issue, 2013–2020.